As will be observed in the first group of 17 cases, 12, or 70.5 per cent., complied with the rule, while in the second group of 14 cases, 8, or 57.1 per cent., were positive, thus agreeing with the rule. Of the total of the two groups, 31 cases, 20, or 64.5 per cent., verified the observations of Gibson.

A reasonable explanation for four of the five discrepancies in the first group is to be found in the presence of a nephritis in three and a myocarditis and obesity in the fourth.

In the second group, of the 6 deviations from the rule, 3 were comparatively young, with soft elastic vessels, in which presumably a relatively lower pressure existed before the onset of the pneumonia, or, at least, were able to withstand a greater depression of the circulation.

Excluding the 7 cases in which a reasonable exception existed, of the remaining 24 only 4 failed to comply with the rule.

Aside from the prognostic significance, the blood-pressure and pulse ratio have been of the greatest assistance and satisfaction as a guide to the administration of cardiac stimulants. In some no stimulation was used, while in others one or two intravenous injections of digitalis were sufficient to reduce the pulse-rate, increase the blood-pressure, and produce a crossing of the curves.

REGENERATION OF BONE.[1]

By Franklin D. Smith, M.D.,
CHICAGO, ILLINOIS.

(From the Laboratory of Clinical Pathology of the University Hospital, Chicago, Illinois.)

The regeneration of bone, formerly with reference to the repair of fractures and in the past century to the ultimate fate of the bone transplant, has been a much-debated topic. One of the earliest and foremost investigators in this field of research was the French naturalist Duhamel du Monceau, who, in 1739, called attention to the active role played by the periosteum in the regeneration of bone. But the exhaustive work of the eminent French surgeon Dupuytren, placed the subject upon a scientific basis. Ollier, Barth, Axhausen, and others have presented many valuable contributions to the subject.

Cellular Dynamics. Some idea of cellular dynamics is essential for an adequate understanding of the physiological mechanism of transplanted tissue. The mere removal of a living cellular mass from its regulated supply of assimilative material—the constituents of the blood and lymph—does not mean death to its individual cells. Cellular life may proceed within certain limits without either organic or somatic influence and under proper chemicophysiological environment will retain its inherent vitality long after its enforced separation from living matter. Upon these fundamental physiological factors depend the eventual outcome of all transplanted tissue, bone included. The successful transplantation of tissue is dependent upon the continuance of the cellular life of the transplant, and likewise necessitates the growth of the individual elements or their regeneration. The living cell just before its removal from the donor is undergoing katabiotic and bioplastic activities respectively, dissipation of kinetic energy in the performance of function, and the conversion of kinetic energy into potential energy, *i. e.*, storing up energy in the formation of the complex molecules of the cells—substance growth. We can dismiss the katabiotic activity going on in the cell by merely stating, for lucidity, that these activities are the responses to external stimuli, either "directly or through the intermediation of the nervous system." The length of time the transplanted cell shall continue to functionate will depend upon the amount of potential energy that it carries with it. Highly differentiated cells will have reserved a minimum of potential energy, *i. e.*, the functional demand placed upon the bone cell is so great and hence its bioplastic activity so limited that even in the most favorable environment it (bone cell) could not be expected to regenerate sufficiently to maintain the unity of the graft. Therefore, since the vegetative potentiality of the bone cell is *nil*, the regeneration of the bone must come from a more embryonic cell. "The more we study the differentiated tissues of the body the more it is brought home to us that the fully developed and differentiated cell, as such, exhibits little active multiplication, and that to a very large extent, under normal conditions, the removal of the cell worn out by use is brought about by the presence and reproductive activity of 'mother cells,' of cells, that is, present in the tissues in a relatively undifferentiated form, or, as we are accustomed to term it, of embryonic type." (Adami.) The embryonic type of bone cell is the osteoblast which is everywhere present in true osseous tissue, in great numbers, in the compact periosseous layer, in the Haversian system, the marrow, and endosteal layer. In these cells, growth is at its maximum, function at a minimum; assimilation and growth with only slight functional demands are the distinctive features of their existence. The amount of reserved potential energy of any individual cell is extremely variable, but upon its reserve capacity depends the time limit of existence before new pabulum is supplied. With the establishment of a blood supply compatible with the demands of the graft, from a few hours to many days after the transplantation, the cells are in a more favorable condition for growth than when first transplanted. When existing upon their own reserve store of energy the cells gradually fall below the limit of maximum efficiency, so that as revascularization proceeds the cells are in active demand for new pabulum, stimulation to growth—or, as Weigert states it, "the katabiotic use of material in function removes the obstruction to growth." While the bone cells in the periphery of the graft have sufficient reserve force to maintain their vitality until reënforced by the permeation of the serum of the host, the bone cell encased in its calcified matrix at any distance from the new supply of food will become necrotic along with its ground substance. This calcium saturated ground substance must undergo dissolution before new bone can be laid down, and most likely the dissolved calcium salts are reprecipitated again by the rapidly maturing osteoblasts.

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[1] This article does not refer to the transplantation of compact bone into cancellous bone.

The graft consists of a multicellular mass, each cellular constituent composed of masses of biophores. The magnitude of growth of each unit mass of biophores is determined by two factors: (1) the relation of the area of surface contact of the unit mass of biophores to the intermediate substance (the cytoplasm), and (2) the relation of surface contact of the cytoplasmic mass to the surrounding assimilative material (plasma of the host). There exists a certain ratio between the optimum efficiency of the cells and their surface contact. Not until this maximum ratio is exceeded will there be nuclear division. It is readily conceived that the above relations vary for almost each individual cell of the transplant, since some cells will be more remote from the maximum supply of pabulum than others, and therefore changes in the biophores dominating the cells. Modification of the environment of like biophores, and eventually adaption of these biophores to this environment, constitutes the process of differentiation of the cells; differentiated osteoblasts are mature bone cells. Any gross change in the biophoric material is associated with corresponding alteration in the cytoplasm, histological and physiological modification of the entire cell.

Complexity of Bony Structure. The regeneration of bone is a very complex process. Bone is a tissue containing several important constituents, *i. e.*, the mature bone cell encased in a calcium salt deposit over which it rules; a complex Haversian system containing endothelium, fibroblasts, osteal fibroblasts in all stages of development, lymph spaces, and all the elements of circulating blood. Its matrix is a highly specialized ground substance which is capable of supersaturation with the earthy salts. Its many modes of development only serve to mystify the ultimate structure; probably no tissue in the human body has as many modes of development as bone. The complexity of the structure and development is the one obstacle encountered in the solution of any problem in which bone either alone or in combination with other tissues is involved. In the study of any problem or problems in which bone receives consideration, it is of paramount importance to keep in mind the presence of the osteoblast, which cannot be differentiated morphologically from the ordinary fibroblast, but which can assume the property of either secreting osseomucin or chondromucin, and eventually becoming, during its course of development, a highly differentiated bone cell, surrounded in its mature state by calcareous walls. By virtue of its inherent power, it is the ruler of calcium deposition within the area of its influence, and also retains the power of dispersing it. In the absence of any data, up to the present writing, to the contrary, the mature bone cell is an end-product, incapable of reproducing itself, and in the event of its death the calcareous ground substance, over which its ruling influence prevades, remains "inherently immutable," requiring time and foreign-body giant cells for its removal. Although it is possible to produce division by mitosis of the mature bone cell by incubating *in vitro* on artificial media, this must not be interpreted as being possible to produce new bone. Mitosis is not always synonymous with growth.

DEVELOPMENT OF BONE. In the consideration of the problems of regeneration of bone, it is advisable at this point to merely call attention to the many modes of regeneration and development of bone. In the embryonic state, it may arise either direct from fibrous tissue, in which case it is known as intramembranous bone, or it may develop from cartilage, as an intermediate stage, and is then known as intracartilaginous or endochondral bone. It is never of primary formation, although always derived from mesenchyme. Either method is complex in its development. After it has passed through the developmental stage and true bone is formed, the process of regeneration and repair are far from simple processes. In the repair of fractures, new bone may be formed direct or formed by passing through an intermediate stage of cartilage but eventually, however formed, fills in the space between the fragments. Loss in bony structure, however produced, may demand regeneration of bone for the preservation of the function of the part. This process will vary, depending upon the type of bone upon which such a demand is placed. The space may be filled in by bone formed from the periosteum or from osseous tissue *per se*. Either process will vary with membraniform bone or chondriform bone, while in the latter type of regeneration the cartilage cells may be (*a*) converted into bone cells and the surrounding matrix converted into osseous tissue, or (*b*) through the agency of fibroblasts, layers of young cells are deposited upon the cartilaginous remains. Just what role the marrow plays in the regeneration of bone is a much-disputed topic at present.

THE METAPLASTIC THEORY OF BONE FORMATION. True bone formation in vessel walls, etc.: In this connection, it may be well to pause and inquire into the mode of formation of true bone in heterotopic tissues, such as bloodvessels, the choroid coat of the eye, and in the ossification of laryngeal and tracheal cartilage in advancing age.

For many years the formation of bone in heterotopic tissues was considered a rare incidence. But since the work of Barth (1895), Pollack (1901), Sacerdotti and Frattin (1902), Poscharissky (1905), Bunting (1906), and Brueger and Oppenheim (1908), the formation of true bone, even with its associated cellular marrow (red), is not an uncommon occurrence. W. Harvey (1907), of Toronto, was able to produce true bone in the aorta of rabbits by the application of irritants to the vessel walls. In from two to six months after the application of a 3 per cent. solution of silver nitrate or a 2 per cent. solution of copper sulphate to the outer coat of the vessel wall of the aorta, Harvey was able to demonstrate the formation of bone with its Haversian canals, with bone marrow or bone alone, or the development of osteoid tissue in areas of calcification. A very important question naturally arises as to the mode of development of bone in these unnatural situations. The metastasis or displacement theory of Cohnheim assumes the presence, in these unexpected situations, of misplaced embryonic tissue, which by some unknown force takes on active vegetative development. This theory might be acceptable if it were not for the comparatively common occurrence of such bony tissue, and moreover, the incidence points to a preponderance of such a frequency of occurrence of the process in advanced arteriosclerosis. Pollack found true bone formation adjacent to the calcium deposits in 43 out of 47 cases in which the process was studied. There are two views (Bunting) concerning the formation of bone in heterotopic tissues, *i. e.*, (*a*) that there is a direct metaplasia of connective tissue into bone after the manner of callus formation, and (*b*) that the calcific material is eroded with the formation of vascularized spaces containing tissue cells, some of which take on the function of the osteoblasts and lay down bone, some of the osteoblasts becoming included and forming the bone cell. Quoting from Bunting: "It seems clear, then, that one has here a metaplasia of connective-tissue cells into osteoblasts, for in vessels and heart valves there can be no possibility of periosteal or perichondral sprouts playing a part except in pulmonary foci. As to the stimulus which leads to this metaplasia there seems to be a uniformity of opinion, as might be expected, from the similarity of pictures presented by the different cases." Paul describes the process as consisting of three stages, as follows: (*a*) calcareous degeneration, (*b*) irritation about these areas of degeneration from fracture or other injury leading to inflammatory proliferation, (*c*) ossification in this young proliferating tissue.

Bone has always formed where young connective tissue is adjacent to calcium deposits and the metaplastic process is evidently a result of the chemical stimulus of the calcium salts. Bone cells, marrow cells, and connective-tissue cells are all developed from the undifferentiated mesoblastic cells, but in "adult life they seem to have diverged widely in nature and function." The specialized differentiation of a fibroblast into an osteoblast is a short step, and is dependent upon the nature of the intercellular substance deposited.

To return to the first appearance of true bone in the embryo, it is readily seen that highly differentiated osseous tissue is developed by a process of physiological metaplasia from the primary mesoblastic tissue, mesenchyme accompanied by a deposition of calcium salts. We must not forget that metaplasia is a constant process in the living body, occurring both as a physiological and pathological process, as is seen in the conversion of cartilage into bone and ordinary connective tissue into fat cells.

INDIRECT REGENERATION OF MATURE BONE CELLS. By the regeneration of any tissue is meant the reproduction of that tissue. The ultimate tissue may be produced in either one of two ways, *i. e.*, (*a*) through a process of direct regeneration (*e. g.*, epithelial cell from epithelial cell), or (*b*) by indirect regeneration (*e. g.*, erythrocytes from the mother-cell mononuclear of the marrow, etc.). A bone cell being an end-product cannot regenerate itself but must depend for its constant reproduction upon the indirect regeneration of the osteal fibroblasts with the ultimate formation of true bone cells. This process of indirect regeneration has been the stumbling-block of many researches in this field of experimentation. What is meant when regeneration of bone from bone is referred to? Certainly, regeneration of osseous tissue from osseous tissue does not refer to the reproduction of bone cells by mitosis with the formation of new bone cells and new ground substance with its deposited earthy salts. As far as the direct division of mature bone cells there is no available data, nor even a suggestion at the present time that such a division is possible in the human body. Therefore, any formation of new osseous tissue from osseous tissue must be by the indirect regeneration and development of its osteal fibroblasts into new-formed mature bone cells. Should a bone cell die through a deficiency of its inherent internal assimilation or a deficient supply of pabulum, as may happen in a transplant, the surrounding calcareous matrix over which the bone cell predominates does not undergo constant replacement and absorption, as any living tissue must, but remains unchangeable only to be removed in due course of time by the endothelial leukocytes, osteoclasts, etc. Therefore any new bone that is deposited, as in the case of the transplant, must be derived from the indirect regeneration of the osteal fibroblasts either of the transplant itself or from the host. This does not mean that all transplanted bone dies and that all new bone is laid down by the indirect regeneration of the osteal fibroblasts. Some bone cells are capable of retaining their inherent vitality and internal assimilation and reign supreme over their stipulated area of calcified matrix. However, it must be admitted, whether by analogy or by direct proof, that the entire quota of constituents of any transplant will not live, cannot live—while other constituents, more favorably situated, will be bathed with the necessary pabulum compatible with the retention of its inherent vitality.

SPECIFICITY OF THE OSTEOBLAST. As to the specificity of the osteoblast, it will suffice to say that from the present animal experimentations its specificity is extremely doubtful. Should this view prove erroneous, then it must be considered as a wandering cell in the blood stream, which with our present histological technic cannot be identified as such. The theory of the metaplastic formation of bone, as in the walls of the bloodvessels, has much in its favor.

BONE A LIVING TISSUE. Moreover, bone is a living tissue and not an end-product, as is generally considered. It undergoes both progressive and retrogressive metabolism throughout its life, exactly like any other tissue in the human body. Old bone is continuously resorbed and new bone laid down. Bone stores up calcium, to be drawn upon in time of need just as subcutaneous tissue stores up fat and the liver stores up glycogen.

Any tissue must undergo internal metabolic changes associated with constant renewal, absorption, and repair from the time of its inception as an entity until complete cessation of its activities. These properties seem comparatively simple when tissue, soft and cellular, undergoing direct regeneration, is under consideration, but when a tissue is apparently immutable because of its hard texture and its regeneration being dependent upon the indirect development of a mother cell the complexity of the process, at first sight, is immeasurable. The mechanical resistance of the hard, calcified matrix merely delays the process of interchange between its constituents and is only a matter of time, all processes of intercellular exchange taking place as in any cellular structure in the animated kingdom. Nails and wire used in the treatment of fractures, etc., produces localized absorption of the bone until the equalization of pressure is compatible with the vitality of the part. This process of absorption of bone under very limited pressure is seen in erosion of the ribs and sternum in an aneurysm of the aorta. Osseous tissue is very susceptible to perverted metabolic processes, as is seen in the pathological changes as a result of rickets, syphilis, tuberculosis, and osteomalacia.

GROWTH OF BONE. It will be of some advantage, now, to consider the process or processes of the growth of bone both longitudinally and peripherally. Primarily, the growth of bone is dependent upon the internal secretion of both the pineal and thyroid glands, as is seen in cretinism, gigantism, and acromegaly. Disturbance in the secretion of the pituitary gland has been suggested as an important factor in the lamellar growth seen in osteitis deformans. McCord has definitely proved by feeding pineal gland to young animals, that in some manner it exerts a marked influence on developing bone. Feeding pituitary extract to fracture cases does not influence the time of permanent callous formation nor complete union of the fractured fragments, as has been shown experimentally.

The longitudinal growth of bone takes place at the metaphysis, *i. e.*, the diaphyseal side of the epiphyseal cartilage. In this situation, because of its nature and construction, the osteoblasts receive an abundance of pabulum for the continuance of their vegetative existence and also sufficient space for their proliferation, free from unnecessary crowding of the ultimate individual constituents. The growth of bone in this region is dependent upon the ossifying cartilage, which initiates the process and contains a full quota of all the elements necessary to its future development. If for any reason the cartilage at the epiphysis should undergo a destructive process with the complete elimination of this type of tissue, then epiphyseal proliferation would no longer be possible or at least would be retarded. Under normal conditions the growth of the bone is limited to the metaphysis; however, should the same conditions prevail in any portion of the diaphysis, the osteoblasts in this area would be stimulated to increased vegetative activity with their resultant specialized ground substance and the formation eventually of bone. These conditions are present when a limited length of the entire circumference of the shaft of a bone is removed. The gap will eventually be filled in by the proliferation of the osteoblasts in this region, both from the ends of the bones and from the periosseous layer of osteogenetic cells. Moreover, there will be renewed activity at the metaphysis with the formation of bone pushing the entire shaft toward the defect.

Bone is increased peripherally by subperiosteal osseous new formation, from the activity of the periosseous osteogenetic layer of the compact bone. This view is accepted by all during the period of development, but is contested during adult life.

Brief Résumé of the Literature. While it may seem a repetition of past publications to attempt even a brief *résumé* of the literature, since an attempt has been made to give due attention in a number of recent contributions on the subject, nevertheless the interpretation placed upon each set of experiments and conclusions varies both with the individual's fitness, either merely as a scientist or as a clinician, and with the subject matter under consideration. I shall attempt to limit my *résumé* of each investigator's researches to the statements and conclusions, specifically referring to the regeneration of bone *per se*. No attempt will be made to discuss pro or con the conclusions, but rather to allow each individual, unbiased, to formulate his own deductions.

Axhausen (1907–1909): "The chief source of new bone which replaces the necrotic bone of the transplant is the periosteum, then next in order comes the marrow and endosteum, and third, only in case of implantation into bone defects, the osteogenetic tissues of the implantation site."

Tomita (1908) concludes that new growth of bone comes from the inner layer of cells of the periosteum and from the marrow cells. The cells of the bone itself has no power to form new bone.

Wieder (1908): "In the regeneration of bone all the various elements, viz., periosteum, cortex, endosteum, and marrow, assist in the process." "Cortical bone-forming activity does not manifest itself until after considerable absorption of the cortex has occurred, so that it may be due to the liberation of bone cells which take on new activity and again precipitate the liberated calcium salts in new situations; or it may be due to the opportunity for the ingrowth of endosteum into the widened spaces." "The deeper layers of the periosteum, the endosteum, the tissues lining the Haversian canals, and the bone cells are probably all related or identical tissues exhibiting different activities, because existing under different physical conditions."

Baschkirzew and Petrow (1912): "There remains nothing else possible than to accept the view that the chief source of the regeneration of bone transplanted into soft parts lies in the primary layer of granulation tissue which surrounds the transplant. Bone in different degrees of necrosis seems to exert a specific irritation upon the connective tissue. Bone freed from the periosteum transplanted into soft parts proves themselves capable of regeneration; periosteum is as little necessary as the marrow."

Cotton and Loder (1913): "The essential picture is summarized as follows: Disappearance of the bone cells in the trabeculæ of the transplant and also to some distance in the host. The graft is covered by a layer of endosteal new bone which unites with the endosteal new bone of the host. New bone is formed by the activity of the endosteoblasts in all parts of the graft, centre and periphery."

Lewis (1914): "Cortical bone free from its periosteum, endosteum, and marrow will retain its vitality and proliferative power when subdivided into small fragments and replaced into the tissues. Contact with living bone is unnecessary for the growth of the transplant; larger pieces of bone may be transplanted and remain alive, not being merely grown into by the bone in which they come into contact." "Bone may unite after a fracture, or a space fill in after resection without the aid of any periosteal or bony bridge." "Contradictory results of careful observers can be explained by the variation in one factor, namely, the amount of blood supply which the transplanted bone obtains."

Bancroft (1914), in discussing the process of repair of bone following trauma, states that "one is impressed with the idea that the calcium salts have been laid down upon ordinary granulation tissue in the perivascular spaces; that is, in the area farthest away from the bloodvessels. This is early formation of new bone."

Mayer and Wehner (1914) state "that under favoring conditions many bone cells of the transplant can maintain their vitality until the transplant has become vascularized. The cells of the Haversian canals, particularly of the young bone, possess osteogenetic power provided their vitality is maintained. A greater part of the transplant necrosis, and that these necrotic areas are gradually replaced by new bone derived from the specific osteogenetic cells of the periosteum, endosteum, and Haversian canals. In bone transplants the bone cells showed no new formation of bone. A part of the transplanted bone dies, another part lives until the transplant is vascularized. The dead bone is partly dissolved by the young bone cells, which form new bone at the same time, which is gradually substituted for the old bone and penetrates into the old bone cavities."

Gallie (1914): "Death of the bone graft by the end of the first week. Revascularization of the graft by the second week. That in bone grafts as well as in living bone, bone absorption and bone building are the properties of living bone cells. By the end of the third week the graft is completely covered by new bone."

McWilliams (1914): "Living bone grafts have life inherent in themselves and are capable of permanent growth even when transplanted into soft parts."

Phemister (1914): "Osteogenesis in bone repair occurs from the inner layer of the periosteum, from the endosteum, and to a much less extent from the bone cells and fibrous content of the Haversian canals. The great mass of bone cells being away from the surface and surrounded by an extensive and difficulty permeable calcified matrix gradually undergo necrosis and absorption. A few (bone cells) about the periphery and lining the larger vascular spaces as well as the fibrous elements of the latter may survive and proliferate. A fracture through a transplant unites by callus formed from the surviving cells of the transplant in the vicinity of the fracture."

Macewen (1912): "The vegetative capacity of the bone cell is at least as great as that of the epithelial cell. Diaphyseal bone graft lives and actively proliferates in its new surroundings. That diaphyseal bone is produced by the proliferation of the osteoblasts derived from the preëxisting osseous tissue. As long as the bone cell remains embryonic, it exhibits the power of proliferation; when it reaches maturity it assumes the fixed tissue type and becomes stationary. This period is coincident with the calcareous deposition, and with it the cessation of active regeneration, though its proliferating potentiality still remains. The regeneration of bone is proportionately in inverse ratio to the size of the graft. The vegetative capacity of the bone cell is as great as that of the graft. The vegetative capacity of the bone cell is as great as that of the epithelial cell, and if one grants not only the vitality of the transplanted epithelium, but also its power of extensive proliferation, then, judging by analogy, the bone cell ought to show equal capability of living and growing when transplanted."

That practically all the bone cells of the transplant necrose is supported by Barth, Bonome, Marchand, Saltykow, Axhausen, Frangenheim, Baschkirzew, and Petrow, while the theory that the graft retains its vitality and proliferates is supported by Macewen and McWilliams. Phemister is inclined toward the former view, but states that the bone cells about the periphery of the graft may survive and proliferate. No doubt the process involved is as complicated as the structure is complex, and no one theory will explain the changes in detail.

Method of Study. The studies of the regeneration of bone in this article are based upon the histopathological changes produced at varying periods of time after the operative procedures. The operative procedures were confined entirely to the practical application of the transplant, such as medullary transplantation, either cortex of the crest of the tibia or fibula complete, excluding macroscopic evidence of the periosteum; inlay transplantation, using an osteoplastid containing a full quota of elements, periosteum, endosteum, and compact bone, placed in apposition to its corresponding layers of the host. All experiments were performed on dogs, rabbits, or sheep, constantly employing the isoplastic or homoplastic methods. While these methods of experimentation are not entirely satisfactory, due to the dual process of repair going on simultaneously, *i. e.*, repair of fracture and regeneration of the transplant, nevertheless the exact duplication of the practical application of the problems and the complexity of the structure under consideration is so great that each individual element cannot be studied separately, the method outlined above is not without its favorable points. Many experimenters have endeavored to separate the various constituents of bone by removing periosteum and transplanting each singly to determine its bone-forming capacity. These have been only futile attempts, as it has been shown elsewhere that it is impossible to remove the periosseous layer of osteogenetic cells with the periosteum without at the same time removing a considerable portion of the cortex. Any attempt that has been made has been a recourse to false security and is unscientific.

Four Factors Involved in the Successful Transplantation of Bone. When an osseous transplant is transplanted into bone, either to fill in a loss of substance or to lend mechanical support to the ends of a fractured bone, there are certain chemical, physical, and physiological demands placed upon the transplant itself, and also upon the adjacent tissues of the host, namely:

1. Temporary maintenance of nutrition not lower than the minimum compatible with the retention of the complete internal assimilation of the various elements of the transplant.
2. Permanent establishment of a sufficient pabulum for the transplant, by the revascularization of the Haversian system of the transplant from the necessary constituents both of the host and the transplant.
3. Complete osseous union between the transplant and the host.
4. Eventual metamorphosis of the transplant into an osseous entity complete in its contiguity with the adjacent tissues of the host.

LAW OF FUNCTIONAL ADAPTATION. Bone transplanted into bone, for specific and physiological reasons, subjected to varying stress and strain, will eventually be governed by the same laws as any other tissues under similar physiological demands. Normal tissues react both to their environment and functional requirements, as pointed out by Wilhelm Roux and known as the law of functional adaptation. This law may be stated as follows: The relation of the conformation, size, and architectural structure of any tissue or organ is dependent upon the functional demands placed upon that tissue; also, any variation in either of these demands will produce histological changes in the tissues sufficient to meet these variations. Transplanted tissues are not exempt from these conditions, and bone transplanted into a defect in bone, to supply a physiological demand and to perform specific functions, will undergo specific changes in its structure commensurate with these demands, *i. e.*, those bone cells retaining a sufficient reserve force to overcome the temporary enforced decrease in pabulum will eventually hypertrophy, subsequently take the place of necrotic bone, and finally functionate in their new locality. This hypertrophy of transplanted bone is well shown in those cases where a complete fibular graft is transplanted into the defect in the tibia. In a remarkably short time the fibula will increase in diameter by the circumferential deposition of new lamellæ. The hypertrophy may be either local or general, and is dependent upon the local application of increased strain upon certain portions of the graft, as in the case of muscle pull or stress applied over its entire structure. Certain functional demands upon the graft are necessary for its future welfare, for in those transplants where there is neither physiological nor mechanical function to perform, while the cells will retain their vitality, their proliferative activity will be materially decreased. Transplanted tissues upon which no functional demands are made will gradually diminish in size, as clearly demonstrated by Lewis and Davis in transplanted tendons, and also by Halsted, who demonstrated that parathyroid tissue would undergo absorption unless a partial parathyropriva primarily existed. No doubt these conditions account for the absorption of the osteoplastid transplanted into certain muscles. Lack of functional demands causes atrophy and absorption.

Because of the tendency to use terms involved more or less loosely, an attempt will be made to limit their application to certain specific entities: Does the term bone cell refer only to the mature cell encased in its calcified matrix? If it is employed in such a sense, then we cannot refer to its vegetative capacity. The term should be applied only to the end stage of development of the osteoblast, encased in its calcified matrix, and in this sense it retains its inherent vitality; but as to its "vegetative potentiality," it is very doubtful. It will not be employed as a collective term, including the osteal fibroblast and its subsequent developmental stages. The osteoblast is a derivative of the undifferentiated osteal fibroblast, and represents a stage of differentiation between the fibroblast of the periosteum, endosteum, and Haversian system and the mature bone cell.

THE FATE OF THE BONE TRANSPLANTED DURING INFECTION. The behavior of the transplant in the presence of an infection will be given special attention. The changes brought about in the transplant as a result of an infection will depend (1) primarily upon the virulence of the infecting microörganism, and (2) upon the different elements which constitute the osteoplastid, viz., the presence or absence of the periosteum will materially influence the extension of the infecting agent while the location of the infecting organism may account for non-union or even fracture of the transplant. Moreover, an infection of the marrow substance of a fibula transplant will add a serious complication, necessitating a second operation and detraction from satisfactory end results.

In the case of a fibula transplant there are three distinct sites of infection, *i. e.*, the marrow and either end of the transplant. A mild infection may be taken care of by the serum of the host if occurring at either end of the transplant. The process of repair at either end would be considerably delayed, depending upon the recognition of a suppurative osteomyelitis and the establishment of sufficient drainage. A properly fitting fibula transplant immediately restores the continuity of the bone, and in case of infection also seals up the active microörganism, which may demand drainage in the future. In the presence of an infection of more or less virulence the transplanted tissue, within its sphere of toxic influence, will undergo necrosis. Infection in a tibia (crest) transplant if suppurative will eventually establish a sinus sufficiently large to take care of the necessary drainage. At the line of fracture the process may become localized, and by necrosis and absorption of the transplant in this region may become so weakened as to fracture at this site.

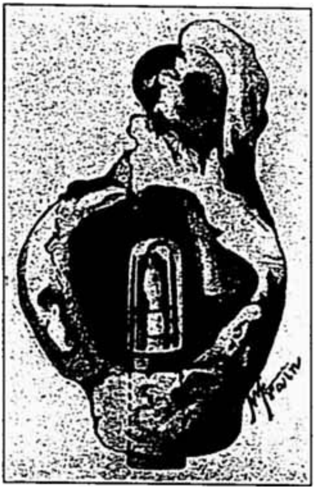
Infection of the transplant with an established sinus does not effect the eventual formation of the external callus, but rather acts as a stimulus to osteogenesis, and an excessive callus is laid down with the immobilization of the fragments. However, an infected transplant will demand removal, since bone dissolution and absorption is very slow, taking months to remove a small transplant.

As the process of infection in other types of transplants, *i.e.*, transplants with the periosteum intact, quoting from Phemister, "these structures (periosteum and medullary substance) may survive and have a reëstablishment of circulation despite the mild infection. The surviving periosteum forms a new layer of bone about the cortex, which both in its excessive amount and in its coarse, spongy character resembles the involurum surrounding the involved shaft in osteomyelitis." "Absorptive changes in the dead cortex are very much accelerated. The surface is eroded, the Haversian canals are dilated, and since deposition of new bone does not keep pace with bone absorption the cortex is rendered somewhat porous in structure."

GLASS-TUBE EXPERIMENT. The humerus of an adult rabbit was cut in two near the condyles and a compact isobone graft was transplanted into the medullary cavity of the distal end, allowing about 2 cm. to project out. Over this free portion of the osteoplastid was placed a glass tube which was sealed at one end and the other end was driven over the distal end of the humerus. The internal diameter of the tube about the transplant was about three times the diameter of the graft. This portion of the tube immediately filled with blood clot, completely surrounding the graft with nutrient plasma. Muscles and hide were sutured with silk. After six weeks the rabbit was bled and the transplant was examined. There was slight proliferation of the osseous tissue about the sawed end of the humerus and also necrosis and absorption about the circumference of humerus from the tight-fitting glass tube. The graft immediately adjacent to the end of the humerus had not increased in diameter, but the free end of the transplant had increased to at least twice its former diameter by circumferential deposition of new bone. While most of the mature bone cells had disappeared, there was evidence of active proliferation of the osteoblasts, especially about the circumference of the distal portion of the transplant.

If transplanted bone (non-infected) is undergoing a process of continuous absorption, as is claimed by some observers, why do we not find more frequently the endothelial leukocyte and the foreign body giant cell—the osteoclasts of Kölliker—for bone absorption cannot proceed by any other process? Osteoclasts in the majority of microscopic sections of transplanted bone must be searched for, and are not nearly as common as the literature would lead us to believe. While it must be admitted that even isobone will undergo absorption in some animals, just as heterobone is absorbed in the majority of untreated animals, nevertheless, it is only the occasional animal in which the isobone transplant is absorbed, and far from the majority. Even heterobone has been known to remain in the human body for years (sheep to man, Drew) and perform its function.

In some cases the absorption of the isobone has been known to be dependent upon the treatment of the osteoplastid. The bed for the transplant must by all means be prepared first and the osteoplastid removed and transplanted immediately, and with very limited manipulation. Small grafts placed in bichloride of mercury (1 to 50,000) for three minutes were always absorbed, likewise grafts were absorbed when the hands were carelessly washed in bichloride (1 to 2000) and rinsed in sterile water. This procedure was at times accompanied by signs of necrosis about the prepared bed. Alcohol (1 to 20,000) for five minutes is extremely injurious to the graft, and its use was always associated with absorption. Freezing, while it has been successful with some tissues, has been unsuccessful in this series of experiments. Heat (110° F.) has been extremely injurious, and has been the source



Transplant in glass tube, six weeks after transplantation.

of considerable trouble in the human transplantation of bone. It is not out of the ordinary to record a temperature of 160° F. taken from the saw immediately after going through three inches of cortex and exercising considerable care. If it is necessary to depend upon the bone cell in the periphery of the graft for its future maintenence, as there are sufficient indications at present to substantiate this view, then it is of paramount importance not to allow the saw to become heated. A few other antiseptic solutions, such as are found in the armamentarium of any well-equipped operating room—mercury cyanide, formaldehyde, etc.—were experimented with. The results were about equal to those cited above.

THE FATE OF THE MEDULLARY ISOBONE TRANSPLANT.[1] *Early Absorption of the Transplant.* Absorption of neither isobone nor heterobone is due to "changed serological relations," as has been claimed by certain observers, since as it has been clearly shown by Lambert and Hanes that transplants are capable of maintaining their vitality, *in vitro*, under artificial incubation, in heteroplasma of a limited variety of species, from which one readily concludes that absorption is not due to a lack of proper food material but rather to some inherent capacity of the cellular elements of the blood or surrounding tissue. Whatever inherent power produces such biochemical differences as to promote autolysis and absorption of the isoplastid, it is certain that the process differs from that which produces absorption of the heteroplastic, the former being dependent upon the bloodvessels and stroma (Bashford) while the latter is dependent upon an immunization process and the action of the macrocytes.

The absorption of the isoplastid, due to sensitization by foreign proteids (production of specific (?) ferments), is one factor which will occasionally account for the disappearance of the graft in a very short time, although in such a delicate process as the transplantation of living tissue, absorption may be brought about by many other factors.

More mature bone cells in the periphery of a graft will retain their vitality than in its central portion. Therefore, more bone cells will retain their vitality per unit of mass of transplant in a complete fibular transplant with two surfaces of the compact bone in contact with the plasma of the host than could be expected in a tibial (crest) transplant with only its external surface in contact with the surrounding plasma. Correspondingly more of the calcium saturated matrix will remain intact, since it is directly under the influence of the mature encased bone cells. The fibulaplastid will require less absorption and replacement than a tibiaplastid, and therefore the surrounding tissue reaction will cease earlier, viz., shorter convalescence.

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[1] These remarks apply only to a periosteal-free compact bone transplant placed in the diaphysis and do not refer to bone transplanted in cancellous tissue in proximity of the epiphysis.

Early Changes Immediately Adjacent to the Transplant. The space between the bone of the host and the osteoplastid where it is not in direct apposition will immediately become filled with coagulated blood and lymph. The clot in time will undergo organization, with the formation of osteoid tissue through which new capillaries will push themselves to the osteoplastid and form a network of fine anastomosing capillaries around its surface. From this vascular network small capillaries are seen to enter the empty Haversian canals.

At either end of the graft a blood clot will be formed, the size of which will depend upon the traumatism to the marrow substance. If the marrow substance has been injured by forcing the transplant against it, producing undue pressure, the changes at the end of the bone are delayed until the proper equalization of pressure is produced by necrosis and absorption. If the marrow has been removed for a sufficient distance, so that the osteoplastid does not produce pressure atrophy and necrosis of the marrow, then both ends of the transplant will be sealed off from the remaining marrow cavity by a blood clot. This occurs within the first twenty-four hours. The endosteum, which has been previously removed from the medullary cavity by curetting, proliferates, covering over denuded bone as far as the blood clot, and later will gradually extend across the concave surface of the clot, sealing off the proximal and distal medullary cavities from the graft by a limiting layer of endosteal cells.

The blood clot at either end of the graft extends farther up on the side of the medullary cavity than it does in the centre, forming a concave surface on the clot.

The blood clot gradually undergoes transformation into osteoid tissue, and in the case of the fibula transplant the osteoid material forms a plug extending into the medullary cavity of the fibula. The transformation of the clot into osteoid material takes place much more quickly than at the sides of the graft, *i. e.,* between it and the host. The calcification of the clot at the ends of the graft progresses quite rapidly, so at the end of thirty days both ends of the graft are solid in the marrow cavity.

The space between the ends of the fractured fragments become filled with clot which finally organizes, but for some unknown reason does not undergo calcification and ossification until many months later. (Approximately seven to nine months in the tibia of a dog, and if conditions are not favorable, this time is materially increased.) This delayed process of calcification is probably due to atrophy of the ends of the fractured fragments extending back from the ends to a varying distance. Not until regeneration has been completed in these atrophied ends does the cicatrix between the fragments undergo calcification and ossification.

This same process of delayed calcification takes place within the medullary cavity, and is especially marked when the transplant is not rounded to fit the medullary cavity. In this the edges of the graft under pressure undergoes necrosis and absorption by the osteoclasts until the pressure is equalized and compatible with the vitality of the surrounding living tissue. While the primary purpose of the medullary transplant is to immobilize the fragments, nevertheless any undue pressure between the transplant and the compact bone of the host will produce a delay in osseous union.

These processes of necrosis and absorption are retrogressive, and are not compatible with regenerative and reparative processes. Not until these retrogressive processes have completely subsided is it possible for any process of a reparative nature to take place. This simply means a delay in a process which is of primary importance, being secondary to immobilization and the proper coaptation of the fragments. Moreover the process of absorption is dependent upon the physical condition of the patient, and is commensurable with the variability of any such process in the human body. The end products of pressure necrosis and degeneration are conducive to excessive callous formation, and consequently detrimental to the complete restoration of function when the fracture is in proximity to a joint, causing limitation of motion.

Any tissue, such as muscle, fat, marrow (?), periosteum, endosteum, etc., which may find lodgement between the compact bone of the host and the transplant will produce a cicatrix which will not become calcified, producing merely a fibrous tissue union.

Finally the conformation of the new osseous development is dependent upon the balanced action of the formative osteoblasts and other cells, osteoclasts which remove any excess of spicules of bony development and mould the bone.

Some of the Haversian canals of the graft become filled with coagulated blood, which will cause necrosis and absorption of the osseous tissue of the area which the canal had supplied. These portions of the Haversian canals do not undergo revascularization, but are eventually replaced by the method of "creeping replacement."

Deposition of calcium in osteoid material at the ends of the transplant must not be mistaken on the interpretation of roentgenograms for absorption of the graft, since serial roentgenograms at weekly intervals demonstrate this area of lessened intensity to be progressive and not retrogressive, as must necessarily be the case in peripheral absorption. Invariably at the end of six months the medullary graft has increased in length and the spindle-shaped ends are due to progressive deposition of calcium in the osteoid material, which is deposited soon after the insertion of the osteoplastid.

Death of the Bone Cells. During the normal metabolic activity of osseous tissue the matured bone cell is constantly being replaced by osteoblasts which have reached the stage of complete maturation. Whether the lacunar space occupied by the wornout bone cell is to be occupied by the new bone cell is not known, although after taking into consideration the complicated structure of the bone cell with its ramifying fibrils extending through the canaliculi it does not seem feasible that such a process of replacement were possible. All the stages of maturation are present at one time during the normal economy of osseous tissue metabolism from the fibroblast to the fully differentiated bone cell. If this is the case in normal bone development we should at least be prepared to accept the same conditions for transplanted bone. In the osteoplastid we find bone cells in all stages of destruction, which continue at a greater rate than in normal osseous tissue. From the second to the tenth day there appears no limit to their destruction. However in each cross-section many living bone cells are always found in the periphery of the graft, while in the central portion the majority will be found shrunken and dead. Many lacunæ are completely devoid of any cellular life, and the staining reaction of the calcified material in its neighborhood is the best guide for the interpretation of the death of the bone cell. Empty lacunæ do not necessarily mean dead bone cells, especially not with our present histological technic. Furthermore it must be remembered that a few bone cells in the host after curetting the endosteum will die, as already shown by Cotton and Loder. Occasionally after a bone cell has taken up its task of producing new bone in the transplant, as shown by the deposition of a few lamellæ of bone, the cell will be found shrunken and the staining reaction deficient. Evidently the bone cell did not have the necessary reserve force to hold out under such a difficult environment. The process of calcium deposition will immediately be assumed by another bone cell. The metabolic interchange in a transplant must necessarily be many times greater than normal to keep up with the increased tissue destruction and absorption.

Reëstablishment of a Permanent Circulation. In normal bone the Haversian canals contain several types of tissues, *i. e.*, the small artery, a large vein, a very narrow lymph channel, and cellular tissue. In the transplant all of these structures are seldom seen. Lymph vessels and veins have not been observed at any age of the transplant. The fine capillary network surrounding the graft, as described above, send fine capillaries into the substance of the transplant wherever it is possible to gain an entrance. No preference is given to the Haversian canals. Occasionally a small capillary has entered the graft only a few microns to the side of the Haversian canal, and shows no indication of preference for the canal. In the fibula transplant the capillaries pass through the compact bone and ramify with the capillaries of the marrow substance. As a rule, most of the Haversian canals show new-formed capillaries, but some are filled up with a coagulated, structureless substance with no indications of bloodvessels.

In constant association with the new-formed capillaries are seen numerous large cells which vary in morphology, and are no doubt maturing bone cells. They are situated on the outside of the vessel wall; sometimes, especially in large Haversian canals, they are in no relation to the bloodvessels, with the exception that they are invariably present. Occasionally these cells have been seen to occupy recesses in the calcified matrix at the side of a small capillary.

The reëstablishment of the permanent circulation is one of the most important stages in the life of the transplant, since not even a beginning of capillary proliferation has been observed in those heteroplastic transplants which sometimes are so readily absorbed. This same process has been seen in heteroplastic transplants taken from the most varied species (pigeon to rat), although a large percentage shows no evidence of capillary proliferation at all.

In the fibula transplant the capillary circulation throughout its marrow substance is readily established, aiding in an early supply of pabulum to the compact bone.

Methods of New Bone Deposition. In any transplanted bone considerable new bone must be laid down to insure the continuity of the graft. In the normal living bone this new bone is almost entirely supplied by lacunar deposition. In transplanted bone, while Axhausen maintains that lacunar absorption and subsequent bone apposition are sufficient to explain the replacement of all new bone, nevertheless, replacement of osseous tissue by direct contiguity—so-called "creeping replacement" (Schleichender Ersatz)—is frequently observed. Bone absorption and bone building are properties of developing and mature bone cells. Bone is absorbed with the liberation of carbonic acid (Hofmeister) setting free the earthy salts for the synthesis of the new bone by the mature bone cells.

The necrotic bone becomes absorbed under the influence of the mature bone cells and new bone is deposited, layer after layer, by these same cells. This process is constantly being observed in all sections of transplanted bone. Occasionally the necrotic bone is replaced by tissue laid down in its immediate vicinity by young bone cells which produce changes in this dead bone by the fibrils which penetrate its substance, and in this way gradually exert sufficient influence over it to produce absorption and deposition of new bone.

Metamorphosis of the Entire Osteoplastid into a Complete Osseous Entity. With the complete reëstablishment of the circulation and deposition of new bone for the necrotic calcium saturated matrix the osteoplastid becomes, after many months, an osseous entity directly continuous with the adjacent osseous tissue of the host. All osseous transplants will not progress to this same degree, since some transplants stimulate osteogenesis to a greater extent than others, producing a large callus sufficient to supply the necessary mechanical demands placed upon it. In such a case the osteoplastic would no longer be required, and its metamorphosis may be discontinued at any stage of the transformation. The time necessary for the complete metamorphosis of the osteoplastid depends upon the size of the graft, condition, and age of the host, and will vary from one to two years.

Final Absorption of the Transplant. The transplant is not a permanent entity, since a gradual decrease in the functional demands is associated eventually with the complete absorption of the graft. This is shown very early in transplants placed in the medullary cavity of the humerus[3] for the repair of recent fractures. In this location in a healthy individual all mechanical demands are removed early with a corresponding early absorption of the osteoplastid. In one case[4] after an excellent result the transplant is undergoing absorption as early as the fourteenth month after operation. Complete absorption will take place in from two to five years, depending upon the size of the graft, age of the patient, and the place of its insertion.

CONCLUSIONS. 1. Osteogenesis is not a specific attribute of any tissue or layer of cells, but is limited entirely to the osteoblasts which are scattered throughout the entire structure of the osteoplastid and the host.

2. Mature bone cells are end products, and while they may undergo mitosis under artificial conditions, this proccess is unknown in the human economy.

3. Many mature bone cells of a transplant remain alive, especially near the periphery of the transplant, and control its surrounding calcified matrix.

4. Absorption of isobone is influenced in many cases by the treatment received by the transplant.

5. Protoplasmic poisons should not be employed during bone-grafting procedures.

6. Regeneration of bone for the most part is an indirect process through the differentiation of the osteoblast to a mature bone cell.

7. A transplant is subject to the varying demands of its environment; functional demands producing an increase in bone deposition; lack of functional demands causes atrophy and absorption.

8. Bony contact is not essential to regeneration of bone, but for practical purposes, doubly insures the result desired.

9. In the transplantation of any bone, the most that can be hoped for is the continued development of the implanted osteoblasts,

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[3] Absorption is not the rule when an entire humerus is replaced by a complete fibula transplant, as in case of sarcoma of the humerus.

[4] Case to be reported by Dr. Charles Davison.

together with such stimulus as may be obtained from the osteoblasts of the host and the retention of vitality in some of the transplanted bone cells with their corresponding intercellular calcium matrix.

10. The transplant in the presence of an infection may or may not survive, and is dependent upon the type of the infecting agent.

11. The medullary transplant is not a permanent entity, but is absorbed as soon as all functional demands are removed.

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